

Chromosome studies in some *Stevia* Cav. (Compositae) species from Southern Brazil

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ABSTRACT

Karyotypes of six species of the genus *Stevia* from Southern Brazil were studied, utilizing root tip metaphases. All species were diploid with $2n = 22$ chromosomes. It was possible to identify each species by chromosome morphology. The basic chromosome number for Brazilian species of *Stevia* is $X = 11$. This number is also found in almost all South American species. We suggest that in *Stevia* there is an evolutionary trend toward chromosomal rearrangement, caused mainly by pericentric inversions. It was found that, in addition to aneuploidy and polyploidy, chromosomal rearrangements are common in the tribe Eupatorieae.

INTRODUCTION

The genus *Stevia* (Eupatorieae, Compositae) has about 230 species distributed from southwestern United States southward through Mexico and Central America. It also occurs from non-Amazonian South America, southward to Central Argentina (King and Robinson, 1987). *Stevia* consists of a group of annual and perennial herbs, subshrubs and shrubs that occur in mountain regions, open forests, borders of rivers, and dry valleys (Robinson, 1930). In Brazil, 36 species have been found, distributed mainly in southern and central regions.

Chromosome numbers have been recorded for the genus *Stevia* (Powell and Turner, 1963; Turner and Flyr, 1966; King and Robinson, 1967; Turner *et al.*, 1967; Coleman, 1968; Grashoff *et al.*, 1972; King *et al.*, 1976;

Jansen *et al.*, 1984; Sundberg *et al.*, 1986; Galiano, 1987; Galiano and Hunziker, 1987; Ralston *et al.*, 1989; Robinson *et al.*, 1989); however, no previous karyomorphological studies have been reported.

MATERIAL AND METHODS

The sources of material and voucher specimens of *Stevia* are given in Table I. The specimens are preserved at the Herbarium FUEL, Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

Metaphase chromosomes were obtained according to methods described by Nogueira *et al.* (1995). The morphological features of the chromosomes include the following: a) absolute length of individual chromosomes measured in μm ; b) relative length represented as the ratio in percentage of the length of individual chromosomes to the total haploid chromosome length; c) arm ratio of each chromosome (long arm/short arm). To determine karyotype asymmetry

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Table I - Origin and voucher number of *Stevia* spp. from Paraná State (PR), Brazil.

Species	Locality of collection	Voucher specimen numbers	Chromosome length (µm)*		TF%*	L/S*	Chromosome type
			Range	HCL			
<i>S. leptophylla</i> Sch. Bip.	Palmeira, PR 25°25'S, 50°01'W	FUEL 10242	1.0 ± 0.06 ^a - 2.1 ± 0.17 ^a	14.6 ± 0.76 ^a	40.9 ± 1.04 ^a	2.2 ± 0.23 ^a	7m + 4sm
<i>S. myriadenia</i> Sch. Bip.	Lapa, PR 25°45'S, 49°43'W	FUEL 10230	1.1 ± 0.08 ^a - 2.4 ± 0.18 ^a	15.9 ± 1.93 ^a	40.7 ± 0.77 ^a	2.1 ± 0.09 ^a	9m + 2sm
<i>S. ophryophylla</i> Rob.	Balsa Nova, PR 25°36'S, 49°39'W	FUEL 09269	1.2 ± 0.11 ^a - 2.4 ± 0.02 ^a	17.3 ± 1.08 ^a	40.6 ± 0.43 ^a	2.0 ± 0.14 ^{ab}	9m + 1sm + 1st
<i>S. rebaudiana</i> Bert.	Paiçandú, Maringá, PR 25°25'S, 51°55'W	FUEL 10232	1.1 ± 0.05 ^a - 2.0 ± 0.18 ^a	16.1 ± 1.35 ^a	39.9 ± 1.48 ^a	1.8 ± 0.05 ^b	7m + 3sm + 1st
<i>S. selloi</i> Sch.	Lapa, PR 25°45'S, 49°43'W	FUEL 09325	1.1 ± 0.10 ^a - 2.1 ± 0.13 ^a	17.1 ± 2.07 ^a	41.1 ± 0.95 ^a	1.9 ± 0.12 ^{ab}	9m + 2sm
<i>S. sp.</i>	Palmeira, PR 25°25'S, 50°01'W		1.1 ± 0.09 ^a - 2.3 ± 0.15 ^a	16.7 ± 1.23 ^a	40.4 ± 1.83 ^a	2.1 ± 0.19 ^a	7m + 4sm

*Means within each column followed by different lowercase letters are significantly different at the 5% level by the Tukey test. Haploid chromosome length (HCL), symmetry index (TF%), and longest/shortest chromosome ratio (L/S).

we calculated the longest/shortest chromosome ratio for each species and the TF% (Huziwara, 1962) where:

$$TF\% = \frac{\text{Sum of short arm lengths}}{\text{Total chromosome length}} \times 100$$

The TF%, longest/shortest chromosome ratios, arm ratios, relative chromosome lengths, and haploid chromosome lengths of all species were compared by one-way analysis of variance and the Tukey test (Steel and Torrie, 1960).

The chromosome number of each species was determined by analyzing at least 10 metaphases obtained from five different plants. We followed the nomenclature of Levan et al. (1964) for chromosome classification where the chromosome types are designated,

according to the position of the centromere, as median (m), submedian (sm), and subterminal (st). Mean chromosome lengths measured in five metaphases of different plants were used for the construction of idiograms.

The methodology of Howell and Black (1980) was used for NOR-banding.

RESULTS AND DISCUSSION

Karyotypic evolution

The karyomorphological data of the taxa studied (Tables I and II) show that the chromosome complement has a similar pattern in all six species. The species are characterized by 2n = 22 chromosomes of re-

Table II - Mean and standard deviation of arm ratio and relative length in the chromosome complement of six *Stevia* species*.

Species	1	2	3	4	5	6	7	8	9	10	11
Arm ratio											
<i>S. leptophylla</i>	1.5 ± 0.07 ^b	1.8 ± 0.15 ^a	1.9 ± 0.15 ^b	1.4 ± 0.13 ^a	1.7 ± 0.11 ^a	1.2 ± 0.11 ^c	1.4 ± 0.13 ^{bc}	1.2 ± 0.07 ^{bc}	1.1 ± 0.05 ^b	2.3 ± 0.18 ^a	1.1 ± 0.03 ^d
<i>S. myriadenia</i>	1.5 ± 0.03 ^b	1.3 ± 0.12 ^d	2.0 ± 0.15 ^b	1.3 ± 0.06 ^{ab}	1.2 ± 0.07 ^c	1.6 ± 0.15 ^a	1.5 ± 0.11 ^b	1.1 ± 0.07 ^c	1.1 ± 0.05 ^b	1.2 ± 0.08 ^c	2.5 ± 0.22 ^b
<i>S. ophryophylla</i>	1.7 ± 0.07 ^a	1.5 ± 0.05 ^c	1.6 ± 0.05 ^c	1.3 ± 0.01 ^{ab}	1.5 ± 0.08 ^b	1.2 ± 0.03 ^c	1.1 ± 0.07 ^d	1.2 ± 0.07 ^{bc}	1.3 ± 0.08 ^a	1.1 ± 0.05 ^c	3.4 ± 0.15 ^a
<i>S. rebaudiana</i>	1.7 ± 0.04 ^a	1.3 ± 0.12 ^d	2.8 ± 0.08 ^a	1.2 ± 0.03 ^b	1.1 ± 0.05 ^c	1.3 ± 0.01 ^{bc}	1.4 ± 0.12 ^{bc}	1.8 ± 0.11 ^a	1.2 ± 0.06 ^{ab}	1.5 ± 0.06 ^b	3.3 ± 0.14 ^a
<i>S. selloi</i>	1.3 ± 0.09 ^b	1.6 ± 0.09 ^{bc}	1.6 ± 0.09 ^c	1.4 ± 0.15 ^a	1.4 ± 0.07 ^b	1.4 ± 0.03 ^{ab}	1.7 ± 0.03 ^a	1.3 ± 0.09 ^b	1.2 ± 0.05 ^{ab}	1.2 ± 0.06 ^c	1.7 ± 0.06 ^c
<i>S. sp.</i>	1.8 ± 0.13 ^a	1.7 ± 0.19 ^{ab}	1.5 ± 0.08 ^c	1.3 ± 0.08 ^{ab}	1.7 ± 0.15 ^a	1.3 ± 0.13 ^{bc}	1.3 ± 0.15 ^c	1.4 ± 0.12 ^b	1.2 ± 0.13 ^{ab}	1.3 ± 0.12 ^{bc}	1.9 ± 0.16 ^c
Relative length											
<i>S. leptophylla</i>	13.5 ± 0.50 ^{ab}	12.1 ± 0.31 ^a	10.4 ± 0.31 ^{ab}	9.5 ± 0.35 ^a	8.8 ± 0.15 ^a	8.5 ± 0.25 ^{ab}	8.0 ± 0.26 ^a	7.9 ± 0.30 ^a	7.3 ± 0.47 ^a	6.8 ± 0.44 ^a	6.7 ± 0.40 ^a
<i>S. myriadenia</i>	14.0 ± 0.34 ^a	11.8 ± 1.04 ^a	10.8 ± 0.91 ^a	9.2 ± 0.25 ^a	8.7 ± 0.15 ^a	8.2 ± 0.20 ^b	8.1 ± 0.25 ^a	8.1 ± 0.13 ^a	7.7 ± 0.26 ^a	7.3 ± 0.26 ^a	6.6 ± 0.24
<i>S. ophryophylla</i>	13.1 ± 0.80 ^{ab}	12.2 ± 0.70 ^a	10.1 ± 0.48 ^{ab}	9.5 ± 0.18 ^a	8.8 ± 0.27 ^a	8.6 ± 0.19 ^{ab}	8.4 ± 0.26 ^a	7.7 ± 0.39 ^a	7.9 ± 0.50 ^a	7.1 ± 0.53 ^a	6.6 ± 0.39 ^a
<i>S. rebaudiana</i>	12.6 ± 0.65 ^b	11.5 ± 0.67 ^a	9.6 ± 0.78 ^b	9.2 ± 0.30 ^a	8.9 ± 0.64 ^a	8.5 ± 0.22 ^{ab}	8.5 ± 0.16 ^a	7.2 ± 0.57 ^a	7.9 ± 0.26 ^a	7.1 ± 0.40 ^a	7.1 ± 0.24 ^a
<i>S. selloi</i>	13.1 ± 0.70 ^{ab}	11.2 ± 0.15 ^a	10.2 ± 0.15 ^{ab}	9.6 ± 0.75 ^a	8.9 ± 0.44 ^a	8.8 ± 0.26 ^a	8.2 ± 0.24 ^a	8.1 ± 0.24 ^a	7.8 ± 0.27 ^a	7.4 ± 0.37 ^a	6.6 ± 0.42 ^a
<i>S. sp.</i>	13.5 ± 0.48 ^{ab}	11.2 ± 0.57 ^a	10.4 ± 0.45 ^{ab}	9.4 ± 0.28 ^a	9.1 ± 0.25 ^a	8.4 ± 0.10 ^{ab}	8.2 ± 0.21 ^a	7.9 ± 0.10 ^a	7.6 ± 0.26 ^a	7.2 ± 0.19 ^a	6.8 ± 0.44 ^a

*Means within each column followed by different lowercase letters are significantly different at the 5% level by the Tukey test.

lately small size, ranging from 1.04 ± 0.16 to $2.38 \pm 0.29 \mu\text{m}$ (Figures 1 and 2). A comparison of haploid chromosome length and absolute length of smallest and largest chromosome (Table I) showed no difference among corresponding pairs in all species. Also the relative lengths were quite similar, the only exceptions being chromosome pairs 1, 3, and 6. However, chromosomes 1 and 3 differed only between *S. myriadenia* and *S. rebaudiana* while chromosome 6 differed between *S. myriadenia* and *S. selloi* (Table II). These differences may be explained by a loss or gain of small segments of chromatin during the divergence of these species. The other species exhibited similar relative lengths. The comparative analysis of arm ratios allowed, on the other hand, a characterization of each karyotype (Table II). Every species showed a difference in arm ratios for at least one chromosome pair. Even in the species with the same karyotypic formula, such as *S. leptophylla* and *Stevia* sp. ($7m + 4sm$) and *S. myriadenia* and *S. selloi* ($9m + 2sm$), the sm pairs were located in different positions in the karyotypes. The distinct patterns strongly suggested the occurrence of pericentric inversions as the rule in the divergence of Brazilian species of *Stevia*.

We detected B chromosomes in *S. leptophylla*, which ranged in number from one to four (Figures 1A and 2A). The B chromosomes varied in size from 0.52 to $0.60 \mu\text{m}$, being approximately one-half the size of the smallest chromosome of *S. myriadenia*. B chromosomes have also been found in *S. grisebachiana* (Galiano and Hunziker, 1987) and *S. yaconensis* (Galiano, 1987) from Argentina.

The data presented in this study and in others reported in the literature for 35 more species from Argentina, Bolivia, Brazil, and Colombia (Coleman, 1968, 1970; Jansen *et al.*, 1984; Sundberg *et al.*, 1986; Galiano, 1987; Galiano and Hunziker, 1987; Robinson *et al.*, 1989) show that, with the exception of hexaploid *S. elatior* ($2n = 66$) from Colombia (Jansen *et al.*, 1984), all South American species of *Stevia* studied are diploid. There is a predominance of the basic chromosome number $X = 11$ among *Stevia* species from South America,

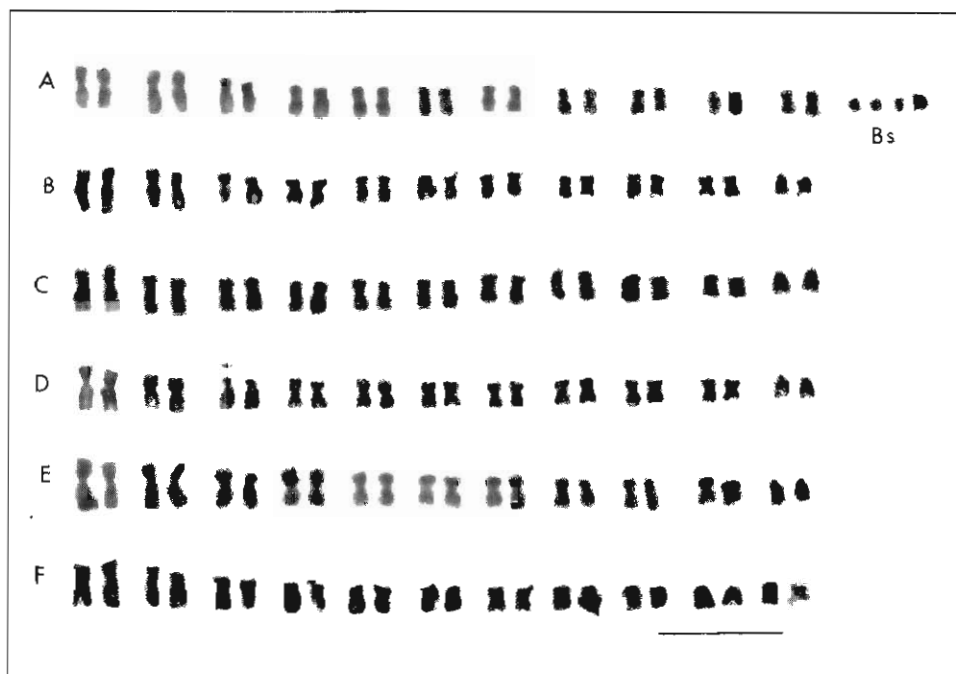


Figure 1 - Karyotypes of six species of *Stevia*. A, *S. leptophylla*, with four B chromosomes; B, *S. myriadenia*; C, *S. ophryophylla*; D, *S. rebaudiana*; E, *S. selloi*; F, *Stevia* sp. (Bar = $5 \mu\text{m}$).

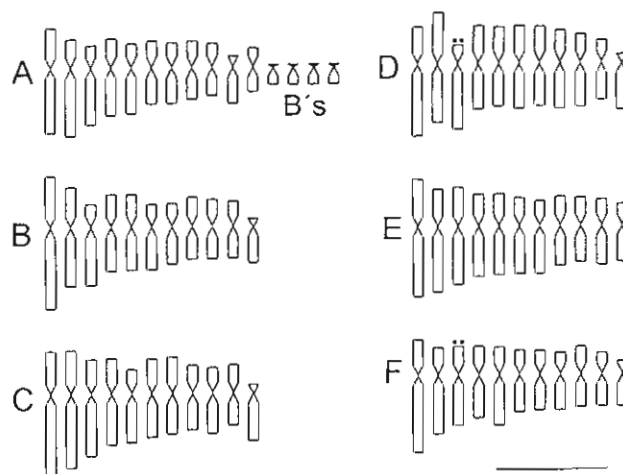


Figure 2 - Comparative idiograms of six species of *Stevia*. A, *S. leptophylla*, with B chromosomes; B, *S. myriadenia*; C, *S. ophryophylla*; D, *S. rebaudiana*; E, *S. selloi*; F, *Stevia* sp. (Bar = $2 \mu\text{m}$).

with only three species (*S. lucida* from Colombia, one population of *S. jujuyensis* from Argentina, and *S. organensis* from Brazil) having $X = 12$ (Coleman, 1968, 1970; Galiano, 1987; Galiano and Hunziker, 1987). These three species may have originated by ascending aneuploidy from species with $X = 11$. From these studies we conclude that the main mechanism in the evolution of the South American species of *Stevia* is probably chromosome inversions, with a small amount of aneuploidy and polyploidy.

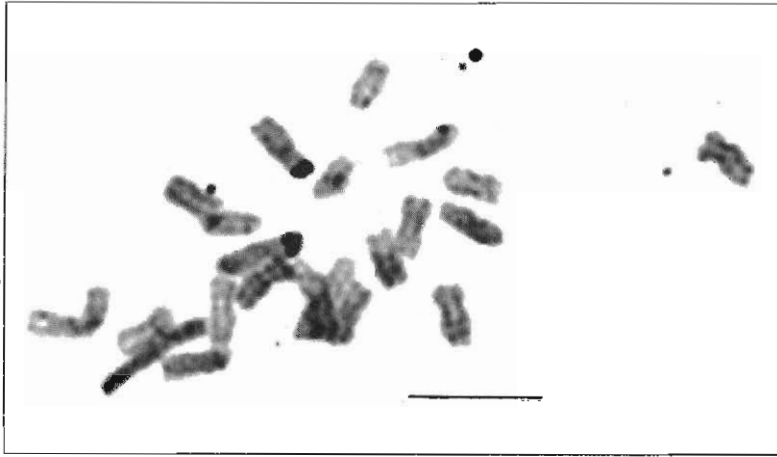


Figure 3 - NOR-banding in *S. rebaudiana* (Bar = 2 μ m).

Studies in other species of *Stevia* have shown that this genus is also widely distributed in the United States, Mexico and Central America, where species with $2n = 34$ seem to be quite common (King *et al.*, 1976). Some North American species with $2n = 34$ and $2n = 33$ are associated with aneuploidy and reproduction by apomixis (Keil and Stuessy, 1975; King *et al.*, 1976; Sundberg *et al.*, 1986). The chromosome numbers $2n = 34$ and $2n = 33$ are probably triploid derivatives of $X = 11$ (King and Robinson, 1987). In the present study we found that $2n = 22$ is common in Brazilian species of *Stevia*, as in other South American species. No evidence of $n = 17$ has been found in any South American species of *Stevia*. Thus, in this region *Stevia* species are typically diploid with a small degree of aneuploidy and polyploidy, while in North American species polyploidy and aneuploidy are common. A similar pattern is observed in the genus *Baccharis* in the tribe Astereae (Ruas, P.M. and Ruas, C.F., unpublished data), where the South American species are exclusively diploid in contrast to a high incidence of polyploidy in North American species.

Small satellites were found on the third chromosome pair of *S. rebaudiana* and *Stevia* sp. (Figures 1D,F, 2D,F). These satellites, due to their small sizes, can be easily lost during preparation; therefore, they are not always detectable. However, the positive reaction to silver impregnation observed in *S. rebaudiana* (Figure 3) confirms the presence of the nucleolar organizer region at the end of the small arm of chromosome 3.

Chromosomal evolution in the tribe Eupatorieae

In a review of the tribe Eupatorieae, King and Robinson (1987) presented a summary of chromosomal evolution in the tribe. However, in addition to the

mechanism of aneuploidy and polyploidy suggested by these authors, other important strategies of chromosomal evolution have been found in the tribe Eupatorieae, including the genera *Mikania* (Ruas and Ruas, 1987; Ruas, 1989) and, as reported in the present study, *Stevia*. In *Mikania* the main evolutionary trend is by aneuploidy and polyploidy with the occurrence of the polybasic chromosome numbers of $X = 17, 18, 19, 20,$ and 21 (Ruas, 1989). In addition, analysis of the karyotypes of 16 species of *Mikania* revealed that species with the same chromosome number are differentiated by structural rearrangements, caused mainly by pericentric inversions. The same mechanism is observed in several cytotypes of *M. micrantha* from Brazil (Ruas, C.F. and Ruas, P.M., unpublished data). In the Brazilian species of *Stevia* it is evident that the main mechanism of chromosomal evolution is also by pericentric inversion. Thus we conclude that, in addition to numeric changes, structural rearrangements have played an important role in the chromosomal evolution of the tribe Eupatorieae.

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RESUMO

Estudo do cariótipo de seis espécies do gênero *Stevia* mostrou que as mesmas são diplóides com $2n = 22$ cromossomos. Pela análise da morfologia cromossômica foi possível a identificação de cada espécie. O número cromossômico básico para as espécies brasileiras é $X = 11$, sendo este o mesmo valor encontrado em quase todas as espécies da América do Sul. Sugere-se que a evolução cromossômica em *Stevia* ocorreu por rearranjos cromossômicos, principalmente por inversões pericêntricas. Verificou-se que na tribo Eupatorieae, além de aneuploidia e poliploidia, a ocorrência de rearranjos cromossômicos é comum.

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